SIMPLE WASHING TECHNIQUE FOR REMOVAL OF PESTICIDES AND HEAVY METALS FROM VEGETABLES AND MUSSELS

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To my beloved mother and father, En. Abd Jalil Bin Lebai Mat and Pn. Rossnani Binti Suboh and to my supportive siblings and special appreciation to Assoc. Prof Dr. Razali Ismail as supervisor.

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ABSTRACT

Washing is a common practice at home and industry to avoid contamination of germs and dangerous diseases and to remove residual contaminants (pesticides and heavy metals) from vegetables. A commercial vegetable washing solution was tested for the removal of nine organophosphate pesticides (dichlorvos, ethoprophos, disulfoton, parathion-methyl, fenchlorphos, chlorpyrifos, prothiofos, azinphos-methyl and malathion) by soaking the vegetables in the washing solution. Meanwhile, in the heavy metals removal study, the samples were washed with sodium acetate chelating solution to remove four heavy metals, namely arsenic, lead, cadmium and nickel. Method validation study was carried out for the analysis of pesticides and heavy metal residues in term of linear range, repeatability, recovery, limits of detection and limits of quantification. The optimum washing practices for pesticides removal were achieved with five minute washing time, 15 mL washing solution and at temperature of 27°C with swirling. The optimized conditions for heavy metals removal were 1 hour chelating time at temperature of 29.5°C and sodium acetate concentration of 500 mg/L. Results showed that six of the pesticides residues were within the safe permitted levels except for ethoprophos (1.4 - 0.15 ppm) and disulfoton (0.25 - 0.03 ppm). Meanwhile, the removal effectiveness for pesticides and heavy metals from the real samples were in the range of 16.7% - 97.3% and 5.5% - 100.0%, respectively. Among the heavy metals studied, only nickel (0.16 ppm) in long beans achieved the safe permitted level after washing with sodium acetate solution. The results also suggested that continuous process is a better technique compared to combined or individual process since the continuous process achieved lower residual contaminant levels than the allowed maximum residue limit for certain pesticides and heavy metals.

ABSTRAK

Membasuh adalah amalan biasa di rumah dan industri untuk mengelakkan pencemaran kuman dan penyakit berbahaya dan untuk penyingkiran bahan cemar (sisa racun perosak dan logam berat) dari sayur-sayuran. Sejenis produk larutan basuhan sayur-sayuran komersil telah diuji untuk meningkatkan kadar penyingkiran sembilan racun perosak organofosfat (diklorvos, etoprofos, disulfoton, paration-metil, fenklorfos, klorpirifos, protiofos, azinfos-metill dan malation) dengan merendam sayur-sayuran di dalam larutan basuhan. Sementara itu, dalam kajian penyingkiran logam berat, sampel telah dibasuh dengan larutan pengkelat natrium asetat untuk menyingkirkan empat logam berat iaitu arsenik, plumbum, kadmium dan nikel. Kajian pengesahan kaedah telah dijalankan untuk analisis sisa racun perosak dan logam berat dalam bentuk julat linear, kebolehulangan, perolehan, had pengesanan dan had kuantifikasi. Amalan basuhan yang optimum dicapai bagi penyingkiran sisa racun perosak dengan rendaman selama lima minit, menggunakan 15 mL larutan basuhan dan pada suhu 27°C dengan bantuan pusaran. Keadaan optimum bagi penyingkiran logam berat adalah 1 jam bagi masa pengkelatan pada suhu 29.5°C dan kepekatan natrium asetat 500 mg/L. Keputusan menunjukkan bahawa enam jenis sisa racun perosak adalah pada tahap yang dibenarkan kecuali etoprofos (1.4 - 0.15 ppm) dan disulfoton (0.25 - 0.03 ppm). Sementara itu, keberkesanan penyingkiran racun perosak dan logam berat daripada sampel sebenar adalah dalam julat masing-masing di antara 16.7% - 97.3% dan 5.5% - 100.0%. Di antara logam berat yang dikaji, hanya nikel (0.16 ppm) di dalam kacang panjang mencapai tahap selamat yang dibenarkan selepas dibasuh dengan larutan natrium asetat. Keputusan juga mencadangkan bahawa proses berterusan adalah teknik yang lebih baik berbanding proses gabungan atau individu kerana proses berterusan mencapai tahap pencemar baki yang lebih rendah daripada had baki maksimum yang dibenarkan untuk racun perosak dan logam berat yang tertentu.

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LIST OF ABBREVIATIONS

ACGIH	-	Association Advancing Occupational and Environmental Health
AR	-	Acid Reduction
As	-	Arsenic
Cd	-	Cadmium
CP	-	Chlorpyrifos
DDE	-	1,1-Dichloro-2,2-bis(p-chlorophenyl) ethylene
DDT	-	Dichlorodiphenyltrichloroethane
DOA		Department of Agriculture
E. coli	-	*Escherichia coli*
EFLE	-	Easily and Freely Leachable Fraction
EPA	-	Environmental Protection Agency
FDA	-	Food and Drugs Administrative
GC–ECD		Gas Chromatography - Electron Capture Detector
GC/MS	-	Gas Chromatography/ Mass Spectrometer
Hg	-	Mercury
HPLC-DAD	-	High Performance Liquid Chromatography - Diode-Array Detection
HUS	-	Hemolytic Uremic Syndrome

LC-MS/MS	-	Liquid Chromatography – Mass Spectrometer / Mass Spectrometer
LLE	-	Liquid-liquid Extraction
LODs	-	Limit of Detection
LOQs	-	Limit of Quantification
MCLs	-	Maximum Contaminant Levels
MENGO		Malaysian Environmental NGOs
MRLs	-	Maximum Residue Levels
NIOSH	-	National Institute for Occupational Safety and Health
OO	-	Organic Oxidation
OPP	-	Organophosphorus Pesticide
PAN A	-	Pesticide Action Network Asia and the Pacific
Pb	-	Lead
POPs	-	Persistent Organic Pollutants
RR	-	Resistant fraction
SPE	-	Solid Phase Extraction
SPME	-	Solid Phase Micro Extraction
TWA	-	Time-Weighted Average
U. S.	-	United States
USDA	-	United States Environmental Protection Agency
WHO	-	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background of Study

It is estimated up to 2.7 million lives worldwide are potentially to be saved each year if fruits and vegetables consumption is sufficiently taken (WHO, 2013). As has been highlighted in the report of World Health Organization, most countries were encouraged to conduct health based programs to achieve the target, at least for the consumer to consume about 400 g of fruits and vegetables daily. The reasons for the recommended consumption volume basically for the prevention of several micronutrients deficiencies especially for less develop countries. For example, in United States, the *Let's Move!* Campaign (Torres, 2014) was organized by Department of Education, in conjunction with the USDA and the U. S. Department of Health and Human Services. The campaign was inspired by the fact that the obesity rate among the children in their country has increased three times, compared to over the past 30 years and their expected lifespan is now less than their parents. In 2006, New York City had launched "Move to Fruits and Vegetables" Campaign (Spear, 2007) through their Health Department and New York State Department of Agriculture and Markets. This expansion of initiative was held to increase accessibility to healthy foods amongst low income neighborhood. Similar programs had been conducted in several countries under different names, but it was held to achieve the same goal which is to increase the ease of understanding to healthy foods among low income neighborhood.

When all people in the world start focusing on staying healthy by eating fresh fruits and vegetables, some consumers may be turned down by widespread use of pesticides and high concentration of pesticides residue. Probably, some or most people had been alerted on the exposure of pesticides towards workers and farmers because they directly deal with those compounds. However, the effects of pesticides residue towards consumer were seldom discussed openly in the community discussion or social media. It became an economical issue to consumers whether to buy organic products or not. They may prefer the safer organic produce which does not use pesticides, but the cost is not affordable to lower income groups. There are few cases reported recently on the *E. coli* outbreak in California and Tennessee this year from identified its source which are from salads (Avila, 2013) and fresh cow milk (Beecher, 2013) respectively. As reported in California, from 21 patients, 2 patients were suspected with hemolytic uremic syndrome (HUS) and fatal kidney complication. Elsewhere in India for example, the Consumer Voice fought against pesticides issue at Delhi Court when they found out farmers used pesticide 750 times higher than the European standards (Arvind, 2013). In fact, some pesticides that have been used were internationally banned pesticides and commonly found in vegetables and fruits. The pesticides can cause headaches, affected fertility and even could damage kidney and liver. Besides that, scientist also found that pesticides and other poisonous chemicals that were usually used in growing fruits and vegetables can cause young children at risk of developing cancer in the future (Sanborn *et al., 2012*).

Pesticides had increased in importance in the agricultural field since it can guarantee the growers to have a quality crop when harvesting and may extend its supermarket shelf life. Previously, organic pesticides had been popular to be used for agricultural field. However, as more pesticides being used, the need for stronger and newer synthetic pesticides keep increasing since the insects will produce new generations that are resistant to the current pesticides being used. Collaborative International Pesticides Analytical Council has categorized synthetic pesticides according to their molecular structure (CIPAC, 2009). Three groups of pesticides are

commonly used which are organophosphates, organochlorines and inorganic pesticides. Organophosphates are the most common and widely used synthetic pesticides. Compared to organochlorines and inorganic pesticides, both groups have higher toxicity and its compound break down in a shorter time period after applying it to the crops. These two pesticides; organochlorine and inorganic pesticides can cause detrimental effects on health which result in severe damage to the brain system of animals and human. Organochlorines pesticides do not easily break down like organophosphates. However, according to scientists, the application of organochlorines will cause pesticides residues to remain in the environment for a long period and can be the major cause of cancer. This group of pesticides contains toxic element such as mercury and arsenic (CIPAC, 2009). These dangerous compounds also known as persistent organic pollutants or POPs already exist for so many years in our environment. Their existence in the environment will be longer and may be incorporated into the food chain and their concentration will be magnified.

Shipman (2013) reported 48 - 81% of pesticide residues found in vegetables in the United States from the year 2000 until 2008. In Malaysia, Mazlan and Mumford (2005) had found the highest content of pesticide active ingredients in cabbage; namely indoxacarb, fipronil and chlorpyrifos combined with cypermethrin with EIQ (Environmental Impact Quotient) value of 42.97, 49.50, 52.80 and 54.50 respectively. Zawiyah *et al*. (2007) also found cypermethrin in several types of vegetables samples in the range from 0.16 to 1.48 mg/kg. Once pesticides being applied to crops, several possibilities on distribution path can be expected (Sangaralingam, 2005). Depending on their molecular structure, some of them will eventually break down after application while some of them will remain on surface of the fruits and vegetables, or remain in soil. When it rains, the rainwater will wash some of them into water supplies or groundwater which then recirculates the pesticides in the environment. Volatile pesticides that are easily evaporated will be distributed in our atmosphere and may finally return together with the raindrop. In addition, pesticides may also be distributed when contaminated fruits and vegetables were consumed. Although concrete facts and cases on pesticides

poisoning cannot be confirmed, the possible danger and effect just cannot be ignored. The acute effect will be through accumulation in our body system and can lead to death. In this study, the used of pesticide focused on eight of organophosphorus pesticides because most of them were listed as common pesticide that can be found in vegetables.

Chen *et al.* (2013) suggested ozone treatment for handling hydrophobic pesticides. This method can be considered as innovative technique but the cost to build the instrument will be of economic issues. Other researcher also had tried proposing hydrostatic pressure treatment as one of the alternatives (Iizuka *et al.*, 2013 (Part 1); Iizuka *et al.*, 2013 (Part 2)). But again, the same factor which is cost effective needs to be considered. Zohair had developed analysis of different washing solution to enhance the washing off effect of several pesticides (Zohair, 2001). The solutions involved varied from acidic, neutral and alkaline solution as well as tap water. He found that acidic washing solution is better for organochlorine pesticides removal as compared to tap water. Overall, it can be concluded that most study of pesticide removal need addition of detergent instead of using only tap water (Liang *et al*., 2012; Fernandez *et al*., 2013).

Pesticides were commonly found in vegetables and fruits since its application were important as protection from insects. Pesticides removal technique were proposed in several studies as mentioned earlier which has their various advantages and disadvantages depending on the consumer's condition. Apart from pesticide, heavy metal was also one of the major problems in food business. This problem had been studied by Azelee *et al*. (2013) who proposed heavy metals removal like arsenic (As), lead (Pb), cadmium (Cd), nickel (Ni) and mercury (Hg) from *Perna viridis* using chelation technique. The removal performance obtained were 54.34% (As), 96.79% (Hg), 82.89% (Hg), 75.02% (Cd) and 61.83% (Ni). Mussels are used as biological indicator (Bedford *et al*., 1966) because of the capabilities as filter feeder. Mussels were also found to have various organic compounds (Somchit *et al.*, 2009) and pesticides (Sivalingam, 1982). Since heavy metals were successfully removed using the chelation

technique, the removal potential study of pesticides using the same technique maybe viable.

Pesticides contamination had been seen in various food products as mention earlier especially in vegetables. Besides pesticides residue, heavy metal also had reported as one of the contaminant in vegetables (Garcia *et al.*, 1974; Fang *et al,*. 2014; Kang *et al.*, 2008 and Kanakaraju *et al*., 2007). Unknowingly, all these studies showed that our food products especially vegetables contain various contaminants which lead to consumer's health problems. Therefore, in this study, a consumer product in market which is "Fruit and Vegie Wash" from Australia was tested on its effectiveness in removing pesticides and heavy metals by using method which had been proposed by Wang *et al*. (2013) and Azelee *et al*. (2013).

1.2 Problem of Statement

Nowadays, consumers are moving towards adopting a healthy lifestyle including making sure all the food that they eat is not contaminated. Washing using tap water is the most commonly used method in our community. However, as reported by Geetanjali *et al.* (2009), washing by using various type of solution had gave difference results in term of dissipation percentage of pesticides level. Although the types of pesticides removed were same which are hexachlorobenzene (HCB), lindane, p,p-dichlorodiphenyltrichloroethane (p,p-DDT), dimethoate, profenofos and pirimiphos-methyl, but this study had shown that the percentage of pesticides dissipation can be increased by adding acetic acid or sodium chloride into tap water. Table 1.1 below shows the percentage of pesticides residue dissipation review of different washing solutions towards tomatoes sample.

Table 1.1: Review on percentage of residue dissipation (Geetanjali *et al.*, 2009).

Processing	Commodity	Pesticide	Initial residues (ppm)	% Residue dissipation
Washing	Tomatoes	HCB	1	51.3
Acetic acid		Lindane	1	47.0
solution		P,p-DDT	1	33.7
		Dimethoate	1	91.5
		Profenofos	1	86.0
		Pirimiphos-methyl	1	93.7
Sodium		HCB	1	42.9
chloride (at		Lindane	1	46.1
10% NaCl)		P,p-DDT	1	27.2
		Dimethoate	1	90.8
		Profenofos	1	82.4
		Pirimiphos-methyl	1	91.4
Tap water		HCB	1	9.62
		Lindane	1	15.3
		P,p-DDT	1	9.17
		Dimethoate	1	18.8
		Profenofos	1	22.7
		Pirimiphos-methyl	1	16.2

In Malaysia, a commercial consumer product from Australia claimed to be effective in removing pesticides. This product was available in several hypermarkets in Malaysia such as Isetan and Jaya Jusco Supermarket. However, the response from consumers was not encouraging. This situation was probably caused by the low level of awareness from Malaysian citizens on the culture of healthy lifestyle, expensive price and insufficient promotion from the manufacturer.

Other than current commercial products in the markets, there are many tips or homemade recipes which have been shared through social medium. They used blogs, website and YouTube as medium to share their recipes on preparation of washing solution to remove any harmful bacteria and also pesticides residue. Usually, the

ingredients used are common kitchen goods such as lemon juice, baking soda, vinegar and salt. These practices have gain lots of positive feedback from consumer since the cost is cheaper and the formulation is easy to be prepared. However, the effectiveness of this technique does not proven scientifically since no study had been carried out based on these homemade recipes. These practices can be nurtured among Malaysian citizens since it may give benefits towards a healthier life. Unknowingly, most population may be exposed to various pesticides through consumption of fruits and vegetables, the pesticide residues tend to accumulate in the body. Concerns have been raised about the possible role of continuous low-dosage exposure in causing certain cancers.

The study on removal of heavy metals from mussel samples have been successfully carried out in 2013 (Azelee *et al*., 2013). The successful chelation technique was tested to study the possibility of removing pesticides from food products such as vegetables and mussels. Finally, this study was further continued to develop combine methods to analyse the performance of pesticide and heavy metals removal either using both methods simultaneously or through continuous washing process and chelation technique.

1.3 Objectives of Study

The objectives of the study are as follows:

i. To determine percentage reduction of pesticides in vegetable and mussel samples using commercial washing solution through washing treatment.
ii. To optimize the washing and chelating treatment conditions for optimum removal of pesticides and heavy metals.
iii. To evaluate levels of pesticide and heavy metal residues via the simultaneous and continuous of commercial washing solution and sodium acetate chelating solution.

1.4 Scope of Study

Washing technique was applied to long beans, broccoli, lettuce, cucumber, tomatoes and mussel samples. The selection of various vegetables is based on different types of skin surface and among the vegetables that are often eaten as raw or made into salad. Mussels represent food sample from marine group. Azinphos-methyl, chlorpyrifos, dichlorvos, disulfoton, ethoprophos, fenchlorphos, parathion-methyl, prothiofos and malathion are chosen for this study because they were listed as the frequent pesticides used by the farmers in Malaysia (DOA, 2014). Removal of metals include arsenic (As), lead (Pb), cadmium (Cd) and nickel (Ni) which are the common toxic heavy metals by using ammonium acetate as chelating solution. Several normal home practices were optimized during washing technique with focus on washing solution, washing time, temperature and swirl effect to achieve maximum removal rate of pesticides. The extract of sample for pesticides was analysed using gas chromatography that equipped with electron capture detector (GC-ECD) whereas analysis of metal using inductively coupled plasma optical emission spectrometry (ICP-OES). Simultaneous removal of pesticides and heavy metal was studied using a proposed design with the expected percentage of removal performances.

In previous studies, many techniques have been proposed as pesticide and heavy metals residues removal. Some of the studied parameters in previous studies of washing were evaluated in this study including time, volume, temperature and swirl effect. However, ultrasonic effect was not included in this study because ultrasonic equipment is rare to be found in normal household appliances. This study was developed according to normal and common practices of consumer during the preparation of food.

1.5 Significant of Study

Compared to hydrostatic treatment, ozone treatment or washing with tap water, washing with commercial solution provides several benefits. This technique is simple with better performance in removing pesticides. It is practical to be done by users without specific skills or equipment. Washing technique maintains the freshness of food products without any adverse effect to human health. Besides that, this study provided a scientific explanation on the suitable conditions for washing method to achieve maximum rate of pesticides and heavy metals removal. This study is hoped to provide assurance for consumer rather than became just as traditional remedies only. In fact, this study can help in exporting market of mussels and other food product, since the level of pesticides and heavy metals can conform to the standard for human food consumption.

From the economic view, the commercial product used in this study provides several advantages to consumers. The market price for this product in Malaysia is about RM 40.00 for a bottle of 750 mL of concentrated washing solution. Normal volume per usage is about 5 mL in 1000 mL of tap water. It can be estimated that one bottle of this commercial product can maximally use for about 150 times which equal to about 5 months. Consumer only spending their money about RM 0.27 for every daily use. In comparison, consumer will face various prices if they prefer in using homemade remedies such as vinegar (RM 1.99/330 mL), salt (RM 1.90/800gm) and lemon (RM 1.50/pcs). This study purposely developed to satisfy and meet the needs of busy consumers, particularly for users with careers because this product is easily to be used by only mixing with tap water. This comparison of economic price is for usage with soaking style. If the consumer prefer in washing with spraying style, a bottle of this commercial product will be last for more than 5 months. Hence, more benefits are gain by the consumer.

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